## REMARKS

For the convenience of the Examiner, this Reply is organized under appropriate subheadings.

Amendments to Claims 52, 90, 101, 102 and 104 and New Claim 105

Claims 52, 90, 101, 102 and 104 have been amended and new Claim 105 has been added to more clearly define that which Applicants regard as the invention. Support for amendments to Claims 52, 90, 101, 102 and 104 and new Claim 105 can be found throughout the specification and the claims as originally filed.

For example, page 20, lines 5-7 describe use of miR15 gene products to inhibit the growth of prostate cancer cells; page 26, lines 5-8 describe isolation of prostate cancer cells from a subject, transfection of those cells with a nucleic acid encoding a miR15 gene product and reintroduction of the transfected cells into the subject; and page 28, lines 3-5 describe administration of a miR15 gene product by direct injection into a tumor, thereby providing support for amendments to Claim 52.

Page 23, lines 9-11 describe a prostate cancer cell as including a cell located in the prostate, as well as a cell from a metastatic tumor of prostate origin, thereby providing support for amendments to Claims 90 and 101 and new Claim 105.

Claims 101 and 104 have been amended to depend from Claim 52, due to the cancellation of Claims 100 and 103, respectively.

Page 27, lines 4-6 describe suitable numbers of transfected cells for introduction into a subject, thereby providing support for amendments to Claim 102.

Amendments to Claims 52, 90, 101, 102 and 104 and new Claim 105 do not add new matter. Entry is requested.

Rejection of Claims 16, 17, 75-88, 95 and 96 under 35 U.S.C. § 112, First Paragraph

Claims 16, 17, 75-88, 95 and 96 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. In support of the rejection, the Examiner cited *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1986) and *In re Wands*, 8 USPQ2d

1400 (Fed. Cir. 1988). The Examiner stated that it would require undue experimentation for one of skill in the art to make and use the claimed invention in light of the breadth of the claims, the unpredictability of gene therapy, the lack of knowledge at the effective filing date of the application regarding the function of microRNAs and the lack of sufficient guidance in the specification. The Examiner stated that there is no evidence that any cancer cell is able to target any cancer site from any administered site in a patient. The Examiner further stated that it is unclear how the miR15 gene product, as an intracellular product, can exert its effects on cells at a site different than the delivery site. In addition, the Examiner questioned how adeno-associated viral vectors, which are replication defective, can deliver the miR15 gene product to other cancer cells. The Examiner also stated that the specification fails to provide sufficient guidance for a skilled artisan on how to overcome the vigorous host rejection reactions against non-autologous transfected cells.

As a preliminary matter, Applicants note that the Disposition of Claims Section in the Office Action Summary indicates that Claims 52, 89-94 and 97-104 were rejected. The rejection in the Office Action under 35 U.S.C. § 112, first paragraph, does not refer to Claims 52, 89-94 and 97-104. Applicants request clarification of the status of Claims 52, 89-94 and 97-104.

In an embodiment, Applicants' claimed invention, as set forth in independent Claim 52, as amended, is directed to a method of treating a prostate cancer tumor in a subject, comprising the step of administering to the subject a cell that has been isolated from the subject and transfected with a nucleic acid that comprises a nucleotide sequence encoding a miR15 gene product, wherein the cell is administered to the subject by direct injection into the tumor.

Dependent Claims 89-97 and 101-105 further limit independent Claim 52 by, *inter alia*, the vector employed, the number of cells administered and the type of tumor treated, in the method of Claim 52.

Applicants have provided substantial guidance in the specification to enable one skilled in the art to isolate a cell from a subject, transfect the isolated cell with a nucleic acid comprising a nucleotide sequence encoding a miR15 gene product and administer the transfected cell by direct injection into a prostate cancer tumor in a subject, as set forth in pending Claim 52, as amended. For example, as previously discussed in the Amendment filed on October 3, 2007, page 13, lines 13-29 and Figure 1a describe miR15 gene products and sequences of miR15 gene

products, such as SEQ ID NOS: 1 and 3; page 25, line 23 through page 26, line 4; and page 43, line 1 through page 46, line 2 describe transfection of cells with nucleic acids encoding miR15 gene products and well established techniques to transfect cells; and page 48, lines 6-25 describe methods to inhibit prostate tumor growth with, *inter alia*, a miR15 gene product.

In addition, page 17, line 3 through page 20, line 4; and page 43, lines 1-29 describe suitable vectors, including recombinant plasmid vectors and recombinant viral vectors, such as adenoviral vectors, adeno-associated viral vectors, herpes viral vectors, retroviral vectors, including lentiviral vectors, Rhabdoviral vectors, and murine leukemia virus vectors, for use in Applicants' claimed methods.

Further, page 26, lines 5-8 describe isolation of prostate cancer cells from a subject for use in Applicants' claimed methods, transfection of those cells with a nucleic acid encoding a miR15 gene product and reintroduction of the transfected cells into the subject. Page 22, line 23 through page 23, line 2; and page 43, lines 1-29 describe features, characteristics and methods of identifying prostate cancer cells for use in Applicants' claimed methods. Page 27, line 24 through page 28, line 5 describe parenteral administration routes of administering the cells employed in Applicants' claimed methods, as amended, including intra-tissue injection, such as intra-tumoral injection. Therefore, Applicants' specification provides guidance for Applicants' claimed method that includes treating a prostate cancer tumor by direct injection of cells obtained from the subject (autologous cells) into the prostate cancer tumor.

In response to the Examiner's question as to how recombinant adeno-associated viral (AAV) vectors, which are replication defective, can yield a therapeutic effect, AAV vectors are known to be useful for expression of genes and have been shown to yield therapeutic effects, as described, for example, by Flotte *et al.*, *Proc. Natl. Acad. Sci. USA 90*: 10613-10617 (1993) (hereinafter "Flotte") and Kessler *et al.*, *Proc. Natl. Acad. Sci. USA 93*: 14082-14087 (1996) (hereinafter "Kessler"), attached as Exhibits A and B, respectively. For example, in the Discussion section on page 10616, Flotte states:

> The experimental models described here demonstrate the efficacy of AAV vectors for expression in primary human airway cells and for *in vivo* gene transfer. The presence and expression of vector genomes in cells from the airway surface epithelium were seen as early as 3 days after [AAV] infection in the rabbits and persisted

> for at least 6 months. These findings indicate that the AAV vectors will be capable of long-term expression after *in vivo* gene transfer to the airway epithelium, an advantage for CF gene therapy.

In addition, in the Discussion section on page 14085, Kessler states:

> Our results demonstrate that AAV vectors efficiently transduce skeletal muscle, resulting in long-term, stable protein expression. The dose-response of AAV-Epo was linear over a broad range of input vector. Lower doses resulted in systemic protein levels which would be within the therapeutic range for Epo delivered as recombinant protein. Higher doses, still on the linear portion of the dose-response curve, may be useful for the delivery of proteins requiring greater therapeutic concentration or with a short half-life.

Thus, AAV vectors are suitable vectors for therapy and induce stable expression of nucleic acids in cells.

In response to the Examiner's statement that there is no evidence suggesting or indicating diffusion of microRNAs from cells, Valadi *et al.*, *Nat. Cell Biol.* *9*(6): 654-659 (2007) (hereinafter "Valadi"), attached as Exhibit C, shows that microRNAs, including miR15, are transferred between animal cells *in vivo* by exosomes, which are vesicles of endocytic origin that are released by many cells, including tumor cells. For example, in the Abstract on page 654, Valadi states:

> Quality control RNA analysis of total RNA derived from exosomes also revealed presence of small RNAs, including microRNAs. The RNA from mast cell exosomes is transferable to other mouse and human mast cells. After transfer of mouse exosomal RNA to human mast cells, new mouse proteins were found in the recipient cells, indicating that transferred exosomal mRNA can be translated after entering another cell. In summary, we show that exosomes contain both mRNA and microRNA, which can be delivered to another cell, and can be functional in this new location.

In addition, on page 657, Valadi states:

> In this study, we have identified different miRNAs, including *let-7*, *miR-1*, ***miR-15***, *miR-16*, *miR-181* and *miR-375* in exosomes... In

> view of the extensive regulatory capacity of miRNAs, the finding that exosomal RNA can be shuttled between cells (Fig. 3h) suggests a novel mechanism by which mast cells may regulate the activity or differentiation of other mast cells. Collectively, the data suggest that the transfer of exosome-derived unique miRNAs or novel mRNAs to recipient cells is a previously undescribed mechanism allowing gene-based communication between mammalian cells. (Emphasis added).

Contrary to the Examiner's statement that there is no evidence suggesting or indicating diffusion of microRNAs from cells, Valadi shows that microRNAs, specifically miR15, can be transferred between cells *in vivo*.

By employing known techniques and the guidance of Applicants' specification, one skilled in the art would be able to isolate a cell from a subject, transfect the isolated cell with a nucleic acid encoding a miR15 gene product and re-introduce the transfected cell to the subject by direct injection into the tumor, as set forth in Claims 52, 90, 101, 102 and 104, as amended, Claims 89 and 91-97 and new Claim 105, without undue experimentation.

## SUMMARY AND CONCLUSION

The specification enables one of skill in the art to make and use the invention, as set forth in Claims 52, 90, 101, 102 and 104, as amended, Claims 89 and 91-97 and new Claim 105 and, thus, meets the requirement of 35 U.S.C. § 112, first paragraph. Therefore, Applicants respectfully request reconsideration and allowance of the claims under reconsideration. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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